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Pharmacokinetics of Scopoletin after Intravenous Administration in Rabbits

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ABSTRACT

We developed a simple, reliable and practical HPLC (high performance liquid chromatography) method to quantify scopoletin in plasma. The experimental analysis was conducted using a reverse-phase C_{18} endcapped column with a mobile phase of CH_3OH-H_2O, 50:50 v/v, pH 2.5-2.8 at a flow rate of 1.0 mL/min. The detection wavelength was set at 340 nm for scopoletin and 260 nm for the internal standard (methylparaben). Method validation indicated that the precision and accuracy of this method were satisfactory. The recovery of scopoletin from plasma after deproteinization was excellent. The pharmacokinetic behavior of scopoletin in rabbits after intravenous administration of a single dose (2, 10, or 50 mg/kg) was studied. The plasma scopoletin concentration-time profile after iv administration was well described by a two-compartment model. For the 2, 10 and 50 mg/kg doses, the values of the steady-state volume of distribution were 9.679, 9.105 and 3.568 L and the total body clearance values were 0.322, 0.382 and 0.184 L/min, respectively. These results indicate that the pharmacokinetics of scopoletin in rabbits after iv administration are nonlinear over a 2-50 mg/kg dose range.

Key words: Scopoletin; HPLC; Pharmacokinetics.

INTRODUCTION

Scopoletin (7-hydroxy-6-methoxycoumarin, Fig. 1) is one of the main constituents of some commonly used herbal drugs, including *Simaba multiflora, Bupleurum fruticosum, Lomatis hirsuta, Senra incana* and *Lycium barbarum*.[1-5] Scopoletin and other coumarins can inhibit xanthine oxidase.[6] Scopoletin is also a prostanglandin synthetase inhibitior,

with a potency five times greater than aspirin.[4] Several investigators have reported that scopoletin also possesses antiinflammatory,[7-9] antispasmodic,[10] antimicrobial[11] and hypotensive[12,13] activity.

Recently, pharmacokinetic studies of the active constituents from herbal drugs has been extensively developed. Understanding of the pharmacokinetics of herbal drugs should enhance the likelihood of safe and effective therapy. There is a lack of information, however on the pharmacokinetics of scopoletin. In this study we developed a high-performance liquid chromatographic method for the determination of scopoletin in plasma samples, and applied this method to examine the pharmacokinetic behavior of scopoletin in rabbits after intravenous administration.

MATERIALS AND METHODS

Materials

Scopoletin was purchased from TCI (Tokyo Kasei, Japan), methylparaben from Sigma (Saint Louis MO, USA), and methanol from Ridel-de Haen (Hoechst, Germany). All other reagents and solvents were analytical grade. study.

Animal Studies

Male New Zealand albino rabbits (2-3 kg) were obtained from the Laboratory Animal Center at the China Medical College (Taichung, Taiwan). These animals were housed under controlled conditions (temperature maintained at 24 ± 1 °C with a 12:12 hr light-dark cycle) for at least one week before use. Water and standard laboratory chow were given ad libitum until 24 h before the experiments, at which time only food was withdrawn.

Scopoletin injections were prepared by dissolving 10 mg of scopoletin in 1 mL of sterilized water. Six rabbits were randomly assigned to receive a single iv bolus of scopoletin (2, 10, or 50 mg/kg). A one-week washout period between doses was used. Blood samples (1.25 mL) were collected through a catheter from the left ear vein at 0, 2.5, 5, 10, 15, 20, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, 300, and 360 min after iv administration in heparinized tube. Samples were centrifuged at 3,000 rpm for 20 min. The plasma was

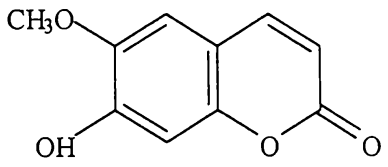

Fig. 1. The chemical structure of scopoletin.

separated and stored at -30 °C until analysis.

Assay Method

The HPLC system consisted of an autosampler (Jasco 851-AS, Japan), two chromatographic pumps (Jasco PU-980, Japan), and an UV detector (Jasco UV-975, Japan). Peak separation was achieved on a C_{18} reversed-phase column (ODS-3, 4.6 × 150 mm, Vercotech Inc., Taiwan). The column efluent was monitored separately at UV 340 nm for scopoletin and 260 nm for methylparaben (internal standard). The programming method for adjustment of the UV wavelength from 340 nm to 260 nm was as follows: 0 min: UV 340 nm → 1.0 min: auto zero → 7.5 min UV 260 nm → 8.0 min: auto zero → 11 min: UV 340 nm. The mobile phase was methanol-water 50:50 v/v (pH 2.5-2.8, adjusted with orthophosphoric acid) at a flow rate of 1 mL/min which was filtered and degassed before use.

A stock solution (1 mg/mL) of scopoletin in methanol was stored at 0 °C. The sample preparation consisted of a clean-up procedure. Two hundreds μL plasma samples were mixed with 800 μL acetonitrile for deproteinization and 15 μl of internal standard solution (10 μg/mL methylparaben in CH_3OH) was added. The mixture was vortexed for 30 sec and centrifuged at 3,000 rpm for 20 min. The supernatant was transferred to another centrifuge tube and dried with a gentle stream of nitrogen gas. Two hundreds μL mobile phase solution was added to dissolve the residue, which was then vortexed and centrifuged again. Fifty μL of the clear supernatant was injected into the HPLC system for analysis.

Two calibration curves were constructed based on the analysis by HPLC of various concentrations of scopoletin (0.01-1 and 1-100 μg/mL) spiked in rabbits plasma. The concentration of scopoletin in rabbit plasma after iv bolus was computed by comparing the peak-area ratio between scopoletin and internal standard of each sample against standard solutions.

Recovery and Precision

Rabbit plasma was spiked with 0.01-100 μg/mL scopoletin. After protein denaturation (with acetonitrile), fixed amounts of methylparaben (as internal standard) were added to the plasma for normalization. The values were compared with the corresponding values of scopoletin and internal standards in vehicles to calculate recovery.

Precision over the entire working dose range was determined by triplicate analyses of plasma samples (n = 3) spiked with nine concentrations (0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50 and 100 μg/mL) of scopoletin. To determine intraday variance, triplicate assays were carried out on the three samples at each sampling time of the day. Interday variance was determined by assaying the spiked samples in triplicate on days 1, 2 and 4. Coefficients of variences (CV) were calculated from these values.

Data and Statistical Analysis

The computer programs JANA and WinNolin (version 1.1, SCI Software Inc., Lexington, KY, USA) were employed to calculate pharmacokinetic parameters:
Fractional rate constant central compartment to out:

$$k_{10} = \alpha\beta/k_{21}$$

Fractional rate constant central to peripheral compartment:

$$k_{12} = \alpha + \beta - k_{21} - k_{10}$$

Fractional rate constant peripheral to central compartment:

$$k_{21} = A\beta + B\alpha/A + B$$

The half-life associated with the macro constant α: α-HL
The half-life associated with the macro constant β: β-HL
The total area under the plasma concentration-time curve:

$$AUC = A/\alpha + B/\beta$$

The total area under the first moment curve:

$$AUMC = A/\alpha^2 + B/\beta^2$$

The total body clearance is the dose divided by the total area under the curve:

$$CL = D_{iv}/(A/\alpha + B/\beta)$$

Mean residence time, MRT, is commonly expressed as the ratio of AUMC-to-AUC:

$$MRT = AUMC/AUC$$

And finally, volume of distribution at steady-state:

$$V_{ss} = MRT \cdot CL$$

Pharmacokinetic parameters are reported as mean ± SD. Statistical analysis of pharmacokinetic parameters estimated at different iv doses (2, 10, 50 mg/kg) was performed by ANOVA repeated measure ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Scopoletin and internal standard were assayed in plasma samples after deproteinization by acetonitrile. The supernatant was dried with nitrogen gas, dissolved in mobile phase and assayed by HPLC. The method was tested and modified to select the best assay. Typical chromatograms are shown in Fig. 2. No discernible noise peaks interfered with the scopoletin and methylparaben peaks. The retention times for scopoletin (340 nm) and internal standard (260 nm) were 5.4 and 9.2 mm, respectively. The detection limit for

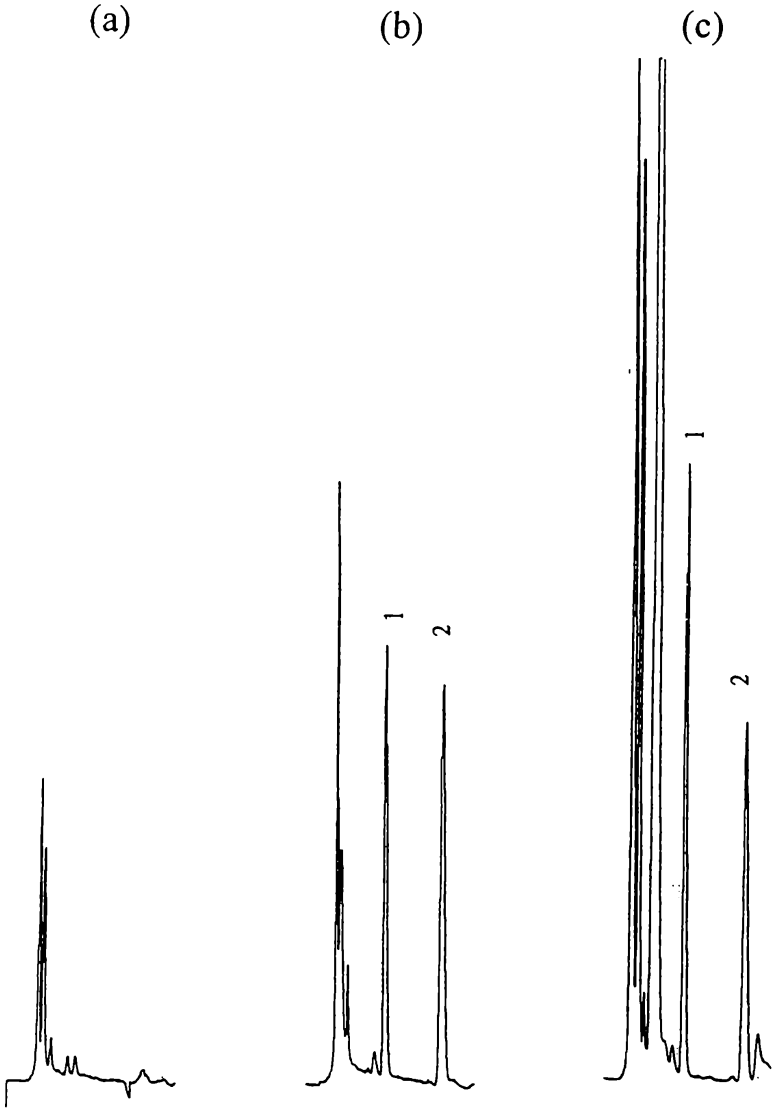

Fig. 2. Chromatograms of scopoletin in rabbits plasmas: (a) blank plasma, (b) blank plasma spiked with scopoletin (10 μg/mL) and internal standard (methylparaben), and (c) plasma sample at 45 min after a 50 mg/kg iv bolus of scopoletin (10 mg/m:). 1, scopoletin (retention time: 5.4 min); 2, methylparaben (retention time: 9.2 min).

scopoletin, at a signal-to-noise ratio of 3, was 10 ng/mL in rabbit plasma. The recovery of scopoletin from rabbit plasma was 91.5-98.6 % for concentrations from 0.01 to 100 μg/mL (Table 1).

The standard curves for scopoletin were made using nine spiked plasma samples over a concentration range of 0.01-100 μg/mL. To cover the concentration range and to increase the sensitivity, it was necessary to use two standard curves. A linear relationship was obtained for both concentration ranges. Coefficients of determination (R^2) were over 0.999 for all concentrations. The regression parameters were $y = 1.2182x + 0.0023$ for 0.01-1 μg/mL and $y = 0.966182x - 0.0003$ for 1-100 μg/mL. y is the peak ratio and the x is concentration in μg/mL.

The reproducibility of the method was be defined by examining both intraday and interday variabilities. The intraday and interday validation studies showed that the coefficients of variation were less than 8.7 % (Table 2). The precision and accuracy of this assay were satisfactory.

The HPLC assay of scopoletin in injections has been described.[14] The pharmacokinetic parameters of coumarin, which has a similar structure to scopoletin, have also been reported.[15] The pharmacokinetics of scopoletin, however, have not been reported. A method to measure the pharmacokinetics of scopoletin after intravenous injection was developed in this study. The results demonstrated a simple, sensitive, reproducible and accurate assay for quantitation of scopoletin in plasma.

Scopoletin concentration in plasma following IV bolus injection is shown in Fig. 3. A

Table 1. Recovery of Scopoletin in Plasma

	Recovery Test (%)		
Spiked conc. (μg/mL)	Scopoletin in plasma (μg/mL)	Scopoletin in methanol (μg/mL)	Recovery
100	97.239 ± 0.664	102.537 ± 1.709	94.863 ± 1.635
50	49.560 ± 0.817	50.268 ± 0.430	98.582 ± 0.788
10	9.639 ± 0.249	10.025 ± 0.077	96.134 ± 2.006
5	4.696 ± 0.071	4.986 ± 0.789	94.189 ± 1.737
1	0.966 ± 0.024	1.037 ± 0.178	93.188 ± 2.478
0.5	0.486 ± 0.009	0.506 ± 0.004	96.035 ± 0.964
0.1	0.100 ± 0.004	0.105 ± 0.002	95.197 ± 2.573
0.05	0.049 ± 0.002	0.052 ± 0.001	94.214 ± 2.575
0.01	0.011 ± 0.001	0.012 ± 0.001	91.508 ± 1.005

% Recovery test: (Scopoletin in plasma/Scopoletin in methanol) × 100 %. Data are expressed as mean ± SD (n = 3).

Table 2. Intraday and Interday Precision for Scopoletin Determination in Rabbit Plasma

Spiked conc. (μg/mL)	Intraday conc. (μg/mL)	CV (%)	Interday conc. (μg/mL)	CV (%)
100	100.702 ± 2.167	2.152	100.717 ± 2.251	2.235
50	49.935 ± 0.101	0.203	49.877 ± 0.253	0.507
10	10.058 ± 0.160	1.587	10.066 ± 0.115	1.138
5	4.991 ± 0.031	0.618	4.998 ± 0.023	0.450
1	1.043 ± 0.062	5.902	1.018 ± 0.037	3.603
0.5	0.505 ± 0.009	1.839	0.520 ± 0.038	7.222
0.1	0.107 ± 0.009	8.566	0.103 ± 0.007	6.444
0.05	0.052 ± 0.004	6.711	0.051 ± 0.002	3.015
0.01	0.013 ± 0.001	8.660	0.011 ± 0.001	5.094

Data are expressed as mean ± SD (n = 3).

two-compartment open model with elimination from the central compartment was proposed and validated to explain the apparent biphasic disposition of scopoletin in plasma after iv bolus. Scopoletin showed a fast distribution and elimination phase. Two h after re-

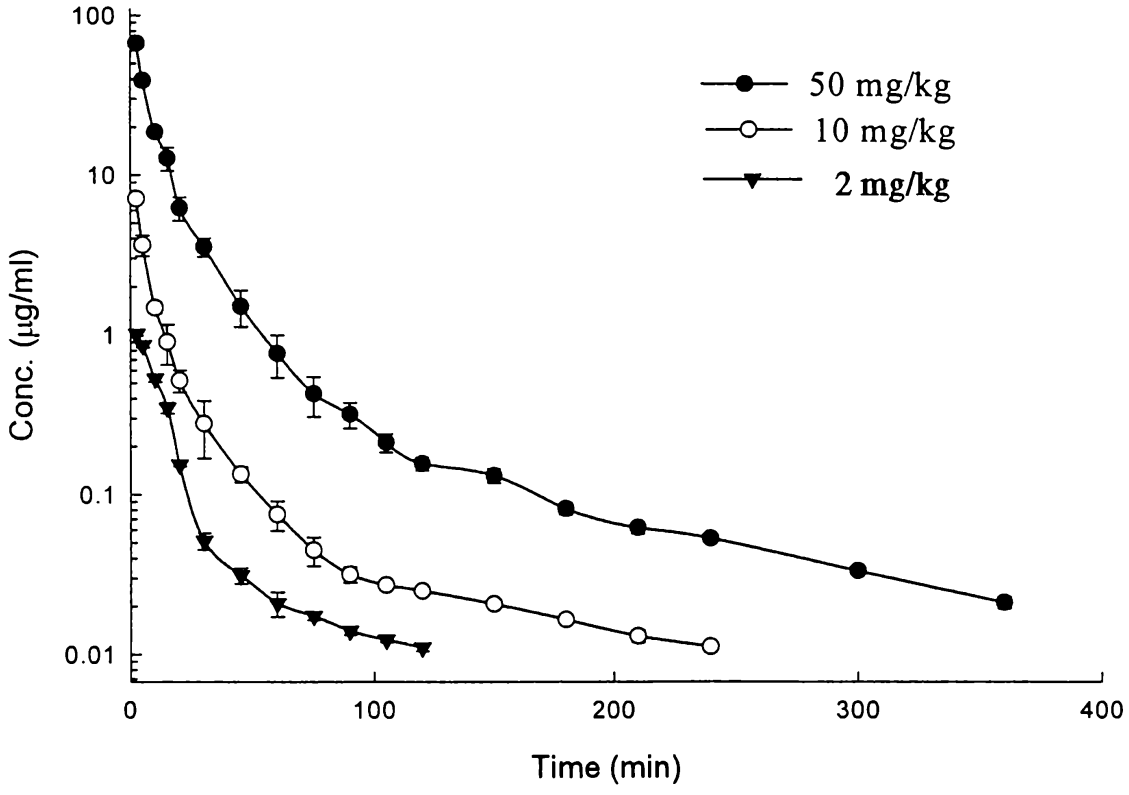

Fig. 3. Mean plasma concentration – time profiles of scopoletin after intravenous administration 2, 10 or 50 mg/kg in six rabbits. Each value represents mean ± SD (n = 6).

ceiving the lowest dose (2 mg/kg), no scopoletin could be detected in plasma. The following equation are applies to a two-compartment open pharmacokinetic model for iv bolus.

$$C = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

In Eq. (1), A and B are the concentration (C) intercepts for fast and slow disposition phases, respectively. The α and β are disposition rate constants for fast and slow disposition phases, respectively.

Analysis of the data after iv bolus (2, 10 or 50 mg/kg) of scopoletin yielded the following equations (2-4), respectively:

$$\text{2 mg/kg: } C = 1.534e^{-0.121t} + 0.035e^{-0.010t} \quad (2)$$

$$\text{10 mg/kg: } C = 6.79e^{-0.125t} + 0.098e^{-0.010t} \quad (3)$$

$$\text{50 mg/kg: } C = 56.756e^{-0.090t} + 0.590e^{-0.010t} \quad (4)$$

The pharmacokinetic parameters obtained by the analysis of these data are summarized in Table 3. Distinct differences were observed in V_{ss}, CL, MRT and AUC values be-

Table 3. Pharmacokinetics Parameters of Scopoletin after IV Bolus of 2, 10 or 50 mg/kg in Rabbits

Parameter (unit)	2 mg/kg	10 mg/kg	50 mg/kg
A(μg/mL)	1.534 ± 0.048	6.794 ± 1.103[a]	56.756 ± 5.511[b]
B(μg/mL)	0.035 ± 0.008	0.098 ± 0.021[a]	0.590 ± 0.083[b]
α(min^{-1})	0.121 ± 0.004	0.125 ± 0.028	0.090 ± 0.011
β(min^{-1})	0.010 ± 0.002	0.010 ± 0.002	0.010 ± 0.000
K_{10}(min^{-1})	0.097 ± 0.004	0.107 ± 0.022	0.083 ± 0.010
K_{12}(min^{-1})	0.022 ± 0.001	0.017 ± 0.005	0.006 ± 0.001[b]
K_{21}(min^{-1})	0.012 ± 0.003	0.011 ± 0.002	0.010 ± 0.000
V_{ss}(L)	9.679 ± 1.403	9.105 ± 1.909	3.568 ± 0.398[b]
α-HL(min)	5.719 ± 0.197	5.781 ± 1.399	7.823 ± 0.953[b]
β-HL(min)	74.301 ± 16.309	74.458 ± 12.830	71.981 ± 2.437
CL(L/min)	0.322 ± 0.014	0.382 ± 0.057[a]	0.184 ± 0.019[b]
AUMC(μg.min^2/mL)	491.213 ± 86.224	1558.420 ± 277.917[a]	13546.00 ± 1630.310[b]
MRT(min)	30.158 ± 4.854	23.838 ± 3.263	19.415 ± 1.404[b]
AUC(μg.min/mL)	16.262 ± 0.287	65.210 ± 5.608[a]	697.172 ± 58.060[b]

Data are expressed as mean ± SD (n = 6).

[a] Significantly different ($p < 0.05$) from 2 mg/kg dose.

[b] Significantly different ($p < 0.05$) from 2 and 10 mg/kg doses.

tween the 50 mg/kg dose and the lower doses ($p < 0.05$). The values of total body clearance were 0.322 ± 0.014, 0.382 ± 0.057 and 0.184 ± 0.019 L/min for 2, 10 and 50 mg/kg bolus doses, respectively. There was a trend towards nonlinearity at a dose of 10 mg/kg, which was not statistically significant; however, between the low (2 or 10 mg/kg) and high (50 mg/kg) doses there was a statistically significant decrease in CL. This parameter is one of the decisive indicators of dose dependence. Saturation of various elimination mechanisms such as metabolism, biliary excretion, and renal excretion may attribute to the nonlinerity of CL.

The values of the steady-state volume of distribution after various doses of scopoletin were 9.679 ± 1.403, 9.105 ± 1.909 and 3.368 ± 0.398 L, respectively. The V_{ss} value after a dose of 50 mg/kg was lower than that after doses of 2 or 10 mg/kg. There was a pattern towards a decrease in V_{ss} that was statistically significant at a dose of 50 mg/kg (Table 3).

The elimination half-lives showed similar values (72-74 min) and no dose dependence. Balanced changes in distribution, clearances, and volumes produced virtually no change in terminal half-lives.

The plasma concentration after a 50 mg/kg dose was relatively higher than after the low doses. The total area under the plasma curves after 2, 10 and 50 mg/kg doses significantly increased with dose. Plasma levels and AUCs disproportionately increased with dose, consistent with dose-dependent kinetics. The differences are particularly clear when the low dose is compared to the high dose. Nonlinear elimination pharmacokinetics are observed in many capacity-limited processes, such as drug metabolism in the liver and transportation of drugs by carrier systems. The kinetics of these processes generally show saturation, i.e. zero-order kinetics, at high concentrations. This study demonstrates that scopoletin is subject to dose-dependent pharmacokinetics.

In conclusion, the high-performance liquid chromatographic procedure with UV detection described in this study is suitable for the quantitation of scopoletin in plasma. The pharmacokinetics of scopoletin after iv administration were nonlinear as evidenced by reduced total clearance, and, steady-state volume of distribution as well as increased area under the curve when a bolus dose was increased to 50 mg/kg. This finding suggests that higher doses of scopoletin may result in non-linear elimination kinetics and also prolongation of its effects.

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